Lipids and Cholesterol Clefs in the Lacunar Cells of Snake Skin

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ABSTRACT

The lacunar cell layer in rat snake epidermis contains many characteristic intracellular vacuoles. The lipid nature of these large round vacuoles was demonstrated by histochemical and ultrastructural investigations. Rhomboid-shaped clefs, similar to cholesterol ester clefs, were observed in proximity to the vacuoles.

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METHODS AND MATERIALS

Skin biopsies were taken from twelve healthy adult Texas rat snakes, Elaphe obsoleta lindheimeri. Formalin fixed tissues were embedded in 2-butoxyethanol glycol methacrylate plastic, and 0.5- to 3.0-μ-thick sections were cut with a Sorvall JB-4A microtome. The plastic sections were mounted and stained with hematoxylin and eosin (H and E). Other formalin fixed tissues were infiltrated in ascending concentrations of gelatin and embedded in 25% gelatin. These blocks were frozen and sectioned at 20- to 50-μ thickness with a freezing microtome. The sections were upgraded to 70% ethyl alcohol, stained with Sudan black B, and mounted on slides.

For examination of cellular ultrastructure, tissues were fixed in 6.25% cacodylate buffered glutaraldehyde, post-fixed in 2% osmium tetroxide, dehydrated rapidly through graded strengths of ethanol, and infiltrated and embedded in Araldite 502 (Ladd Research) or Epon 812 (Polysciences). Mounted ultrathin sections were stained with 2% uranyl acetate and 1% lead citrate and examined with an RCA EMU 4C electron microscope.

RESULTS

The histological examination of the rat snake integument which was embedded in plastic revealed that the epidermis consisted of a multistrata of living and keratinized layers, similar to that described for other species of snakes (Maderson, '65; Downing and Roth, '74; and Jackson and Reno, '75). The lacunar cells were first recognizable in the rat snake integment within the upper intermediate group of cells during the early proliferative phase. At the late proliferative stage, the lacunar cell layer was two to three cells thick, and the lacunar cells near their junction with the alpha keratin layer were large and flattened. The abundant unstained clear vacuoles were of various sizes and filled the cytoplasm (fig. 1). It is presumed that the vacuolar contents were removed during the histological preparation. An irregularly shaped nucleus was strongly basophilic and occupied a central position within the cell.

The examination of frozen sections which were stained with Sudan black B (a lipid stain) revealed that the vacuolar contents of the lacunar cells were intensely stained (fig. 2). The alpha keratin layer was weakly stained, while the remainder of the epidermis and dermis were sudanophobic (fig. 2). Further examination of lacunar cells which were sectioned somewhat obliquely revealed that

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their cytoplasm was filled with numerous stained granules (fig. 2, insert). The granules were round and contained sudanophilic material which was suggestive of stained lipid. The nuclei and other cytoplasm of these cells remained unstained (fig. 2, insert). The examination of the lacunar cells with the electron microscope revealed large round single membrane bounded droplets which contained a homogeneous material and which were distributed throughout the cytoplasm of the lacunar cells (fig. 3). The droplets corresponded in size and shape to the large empty vacuoles seen in the plastic H and E stained sections and also to the sudanophilic granules seen in frozen sections. In addition to these vacuoles, numerous small vesicles and alpha-filaments were present (fig. 3). Lacunar cells next to the alpha keratin layer contained many conspicuous rhomboid-shaped bodies which occupied either a perinuclear or peripheral position within the cytoplasm (figs. 4, 5). As discussed later, such bodies resemble the cholesterol ester clefts seen in tissues rich in lipid and cholesterol. In many lacunar cells, both lipid droplets and cholesterol ester clefts were present (fig. 5).

Lacunar cells in the rat snake gradually matured, keratinized, and contributed to the thickness of the alpha keratin layer. Alpha-filaments were found interspersed throughout the lacunar cytoplasm, and were conspicuously present in the outer lacunar cells (those cells about to be incorporated into the alpha layer) and in positions adherent to desmosomal cell junctions.

**DISCUSSION**

Large vacuoles are a salient feature of the lacunar cells in the Texas rat snake epidermis. The vacuoles described herein appear similar to the vacuoles described in lacunar tissue of other species of snakes by Horstmann (‘64), Maderson (‘65), Roth and Jones (‘67, ’70), Roth and Baden (‘67), Downing and Roth (‘74), Miscalencu (‘74), and Jackson and Reno (‘75). Similar lacunar cells were likewise described for the squamate lizards (Maderson and Licht, ’66; Bryant et al., ’67; and Maderson et al., ’72). Because the vacuoles appear empty in paraffin embedded and H and E stained sections, previous researchers thought that the vacuoles perhaps were formed during cytoplasmic degradation steps (Maderson, ’65; Roth and Jones, ’67). Our histochemical and ultrastructural studies, however, have demonstrated that the larger vacuoles are filled with materials that stain and appear similar to lipids. Apparently the lipid contents in the other studies were extracted by alcohol dehydration procedures during histological preparation, whereas we used gelatin embedded frozen sections which prevented the loss of sudanophilic lipid. A previous histochemical study on snake skin (Goslar, ’58) unfortunately failed to demonstrate lipids in the skin of *Natrix natrix*; however, in a later paper, Goslar (’64) restated those findings with reservation, and noted that additional clarification was needed in snake skin studies.

The presence of lipids and the process of lipogenesis in rat snake skin is intriguing. Lipids which could be arranged in an epidermal layer, such as the lacunar layer, would assist in the inhibition of water loss and solute penetration. In the strictest sense, the lipid rich lacunar layer would be like spreading a layer of vaseline over the skin. Perhaps a phylogenetic implication concerning the evolution of the vertebrate epidermis might be indicated here also, i.e., the evolutionary change of a wet amphibian skin to a dry reptilian integument. Other researchers likewise have reported lipids in reptilian skin. Matoltsy and Huszar (’72) described a relatively large proportion of lipids in turtle horny cells, and they suggested that this revealed a close relationship to the lipid rich avian horny cells. Ultrastructural studies (Matoltsy and Bednarz, ’75) also confirmed the presence of lipids in turtle skin. It is unclear whether lipids in the rat snake were being actively produced within the cells, or if they might have been the products of other cellular processes, such as lamellar membrane degeneration. The presence of smooth vesicles which resembled smooth endoplasmic reticulum of other lipid synthesizing cells, however, suggested an active synthesis of cholesterol and other components of lipids within the lacunar cells. Alpha-filaments are also produced in the lacunar cells. The autoradiographic study by Roth and Baden (’67) demonstrated that protein synthesis in the epidermis of the Indigo snake first began in the lacunar cells during the resting phase and reached a peak of synthesis during the early proliferative stage. The increase in ribosomes in the cytoplasm of the rat snake lacunar cells and additional accumulations of keratin throughout the cells are indicative of
the gradual process of keratinization of these cells prior to their incorporation into the alpha keratin layer.

The demonstration of the needle- or rhombo- boid-shaped bodies in the lacunar cells is puzz- ling. The clefts surely are not the result of artifactual remnants created by the loss of either keratin or pigment granules, and be- cause the clefts appear in various orientations in both epon and araldite embedded specimens and are selectively restricted to the lacunar layer, it seems unlikely that they were pro- duced by microtomy or ultrastructural prepa- ration procedures. Instead we believe that the clefts are the remnants from a crystalline in- clusion which is indigenous to the lacunar cells. Similar rhomboid-shaped structures were shown in other studies of snake epider- mis (Schmidt, '59; Horstmann, '64) and in lizard lacunar cells (Bryant et al., '67). Schmidt ('59) utilized polarized light micros- copy to examine refractive crystalline de- posits (= Kristallblätchen) in the loose horny epidermal layer of python; Schmidt's description and illustrations clearly depict a crystalline nature for the rhomboid clefts. Bryant et al. ('67) commented that the large elongated and elliptic vacuoles in the lizard lacunar cells were empty, while smaller rounded vacuoles were filled with a homoge- neous, relatively translucent material. We contend that the former vacuoles of their study are comparable to the clefts in the rat snake epidermis, while the rounded vacuoles probably are lipid in nature.

Another favorable criterion for the in- digenous crystalline inclusion theory is that the clefts in the rat snake strongly resemble the irregular shaped clefts formed by the cholesterol esters described in rat adrenals (Szabo, '68), human hepatocytes (Lough et al., '70), human gall bladder with cholest erosis (Koga et al., '75), human aortic atheromas (Ghidoni and O'Neal, '67), and atherosclerotic lesions (Geer et al., '61). Similar structures have also been found in experimentally ind- uced cholesterol producing situations (Scal- len and Dietert, '60). Moreover, cholesterol esters have been biochemically associated with epidermal lipids in humans (Downing and Strauss, '74), rats (Freinkel and Fiedler- Weiss, '74), and mouse (Chan and Black, '76).

We are uncertain how or why cholesterol esters occur in lacunar cells of snake; how- ever, because of their proximity to the alpha keratin layer, it is assumed that they could play some part in the keratinization process. Also since the lacunar cell layer is shed with the outer epidermal generation, ecdysis may be a procedure for cholesterol elimination.

LITERATURE CITED


PLATE I

EXPLANATION OF FIGURES

1 Photomicrograph of H and E stained section of rat snake skin. The lacunar cells (Lo) contain numerous vacuoles. Above the lacunar cell layer is the alpha keratin layer (Ao). The stratum germinativum (SG) and dermis (DR) are also seen. × 1,850.

2 Photomicrograph of snake skin sectioned with a freezing microtome at 40 μ thickness and stained with Sudan black B to demonstrate lipid. The lacunar cells (Lo) are strongly sudanophilic, while the alpha keratin layer (Ao) exhibits mild sudanophilia. The beta keratin layer (Bo), other epidermal layers, and dermis are sudanophobic. The insert demonstrates individual sudanophilic lipid droplets (arrowheads) surrounding the unstained nuclei (asterisk) of the lacunar cells. × 1,850 and × 2,000.

3 Electron micrograph of a lacunar cell within the rat snake epidermis. The lacunar cell is characterized by many large lipid droplets (L) which correspond to the droplets seen in figure 2. The nucleus (N) is irregular in outline and is surrounded by numerous small clear vesicles (Ve) and alpha-filaments (F). × 8,500.

4 Electron micrograph of a lacunar cell juxtaposed to the alpha keratin layer (Ao). Numerous cholesterol clefts (CC) surround the nucleus (N) and fill the cytoplasm. Also present in the cytoplasm are alpha-filaments and small vesicles. Desmosomes (D) indicate the cell junctions. × 7,850.

5 Electron micrograph of a lacunar cell near the alpha keratin layer. This lacunar cell contains both the rhomboid-shaped cholesterol clefts (CC) and lipid droplets (L). Small clear vesicles and alpha-filaments are seen in the cytoplasm. The nucleus (N) and desmosomes (D) are labelled. × 10,000.
LIPIDS IN SNAKE SKIN LACUNAR CELLS
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PLATE 1

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